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Assessing zinc in humans

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Abstract

Purpose of Review

To examine the most recent literature that provides new data regarding the potential and emerging biomarkers for zinc status in individuals.

Recent findings

Suboptimal dietary zinc intake is estimated to affect 17% of the world's population, however the assessment of zinc status is notoriously difficult. A systematic review and meta-analysis of studies investigating biochemical biomarkers of zinc status was conducted by the European Micronutrient Recommendations Aligned (EURRECA) network. This review summarised the data published from inception to 2007. More recently (2016), an international expert panel, convened by the Biomarker of Nutrition for Development (BOND) initiative, published an extensive review the literature addressing biomarkers of zinc status in populations and individuals and categorised the biomarkers as useful (dietary intake, serum [Zn], stunting), potentially useful (hair [Zn], urine [Zn], neurobehavioural function), and emerging (nail [Zn], oxidative stress and DNA integrity, zinc kinetics, zinc dependent proteins, taste acuity).

Summary

The most recent data on the potentially useful biomarkers support the further investigation of hair [Zn] and indices of neurological function, particularly those assessing memory and attention. Of the emerging biomarkers, the measurement of DNA integrity and the expression of zinc transport proteins look promising.

Key words: Zinc, biomarker, status

Introduction

Inadequate dietary zinc intake affects 17% of the world's population, and the assessment of zinc status in individuals and populations is key to informing our understanding of zinc homeostasis in health and disease, and to evaluating the efficacy of zinc supplementation programmes. In 2009, a comprehensive systematic review and meta-analysis of zinc biomarkers from inception to 2009 was published by the EURRECA consortium (1) in which 32 potential biomarkers were considered. The meta-analysis revealed that, plasma or serum Zn concentration ([Zn]), hair and urine [Zn] did respond to changes in dietary zinc intake and were useful biomarkers of zinc status, with specific caveats. However, for many biomarkers, there were insufficient data to fully evaluate their potential. More recently complete review of zinc biomarkers was published in a report by the Biomarkers of Nutrition for Development (BOND), zinc expert panel (2). This review considered both biochemical and functional indicators of zinc status, and classified them into four categories; "recommended", "potential", "emerging" and "not useful". A potential biomarker is one that shows promise but data are in-sufficient to establish a specific cut-off indicating zinc inadequacy in populations. These include hair and urinary [Zn], and neuro-behavioural function. Emerging biomarkers are those for which there is some theoretical basis of a relationship to zinc intake or status, but the testing is insufficient to confirm the relationship. These include nail [Zn], zinc-dependent proteins, oxidative stress and DNA integrity, zinc kinetics and taste acuity (table 1).

The purpose of this review is to examine the recently published data regarding the potential and emerging biomarkers for individuals identified by BOND.

Insert Table 1 here.

Review

MEDLINE and EMBASE databases were searched from January 1st 2015 to April 1st 2016 for publications investigating each of the potential and emerging biomarkers listed in table 1. The search

was limited to English language and human studies, full papers. Abstracts and reviews were excluded unless the review included a meta-analysis (3). The papers identified for inclusion in this review are summarised in Table 2.

Insert table 2 here.

Hair [Zn]

Zinc is a structural component of the hair matrix, formed in the follicle. Hair [Zn] therefore reflects the availability of zinc from the blood supply at the time of formation (2). Hair has the advantage of being easily collected and is stable indefinitely if stored appropriately. Included studies collected hair samples ranging from 0.2 to 2g, from the nape of the neck, adjacent to scalp (4, 6), or from 1.5 cm from scalp at the nape or occipital region (5). Hair samples were washed in deionised water, dried and acid digested prior to analysis by either atomic absorption spectrometry (AAS) (4, 5) or inductively couple plasma mass spectrometry (ICPMS) (6).

Children

Gao *et al*. evaluated the relationship between hair [Zn], growth and indices of cognitive development in young children from 13 provinces in China. Anthropometric measures were assessed alongside hair [Zn]. The tenth percentile (P_{10}) of hair [Zn] of children in each age group served as the threshold value, with a value below P_{10} indicating hair zinc deficiency. The P_{10} values for children aged 3-4 years was 47.5 μg/g, and 41.3 μg/g for children aged 4-5 years. Overall 15.46% of children had hair zinc deficiency. There were no significant correlations between hair [Zn] and anthropometry, except for weight for age in boys (p=0.04) but not girls. When separated into 2 groups (below and above threshold P_{10} value), there were no significant differences in anthropometric measures.

A study undertaken in school children in Cuba by de Gier *et al* (5), examined hair [Zn] as a marker of zinc status alongside anthropometric measures, in children with and without soil transmitted

helminth infections (STH) (5). The median (IQR) hair [Zn]s for the STH infected and uninfected children were 113.35 μg/g (94.4-143.7) and 112.55 μg/g (88.3-136.0) respectively. Overall, 12.2 % of the children were deemed to be zinc deficient based on a cut-off value < 70 μg/g ((16). The overall association between hair [Zn] and height for age was not significant but did show a positive trend (p=0.082). When stratified for helminth infection, the uninfected children had a significant positive association (p=0.033) between hair [Zn] and height for age, but there was no association in infected children.

Adults

Koc et al (6) compared hair and serum [Zn] in patients with Alzheimer disease. Hair [Zn] was significantly lower (p=0.02) in patients (75 μg/g ±29) vs control (98 μg/g ±54), however there were no significant differences in serum [Zn] between the Alzheimer patients (0.47 ± 0.1 μg/ml) and healthy controls (0.52 ±0.3 μg/ml).

A systematic review and meta-analysis was performed by Bin Liu *et al*., examining the relationship between [Zn] in hair, serum (or plasma), and myocardial infarction (MI). The meta-analysis of pooled data revealed that both serum [Zn], and hair [Zn] were significantly lower in patients with MI. The lower serum [Zn] in MI patients may be due to the acute phase response, however the authors contend that hair [Zn] represents a longer term measure of the zinc status of the patient, and may indicate an underlying chronic zinc deficiency in MI patients that could be a causal factor in the condition.

The studies in children enabled a comparison between hair [Zn] and growth. However only one of the studies reported a significant association between hair [Zn] and height for age (5). The studies in adults enabled an evaluation of hair zinc alongside serum [Zn]. Both studies reported a fall in hair [Zn] in the patient groups, one without a concurrent fall in serum [Zn], suggesting a possible

advantage in measuring hair over [Zn] for chronic zinc deficiency, and in the presence of the acute phase response.

Neurobehavioral and cognitive function

Zinc plays a key role in neurodevelopment from conception through childhood. In addition, it acts a neurotransmitter, playing a role in learning and memory function throughout life. Five studies were identified that measured indices of zinc status and neurobehavioral and cognitive function, one was conducted in infants (7), two in pre-school aged children (4, 8) and two in older adults (9, 10).

Infants and children

In order to assess the relationship between zinc status and brain development in infants adopted internationally into families in the USA Fuglestad *et al* (7) conducted a comprehensive nutritional and neurodevelopmental assessment at baseline (within one month of arrival) and a six month follow-up. Infants (n=53) raised by biological parents in the USA served as controls to establish the normative data for neurodevelopment. Neurodevelopment assessment included general cognitive and motor development (Bayley Scales of Infant Development-III, BSID-III). In addition, at follow-up, memory function was tested using elicited imitation tests. At baseline, anthropometric measures revealed that 35% of the adopted children were stunted (HAZ<2) and 29% were zinc deficient (serum [Zn] < 60 μg/dl)]. Changes in the rates of deficiencies from baseline to follow-up were not statistically significant, with the exception of a reduction in stunting. Neurological function tests revealed that adoptees with zinc deficiency had significantly poorer performance in the elicited imitation test than those with normal zinc status and those with iron deficiency had lower BSID-III scores than controls. There were no differences in any of the neurodevelopmental measures between the deficient and sufficient groups for vitamin D, vitamin A or iodine/selenium.

Gao *et al* (4) examined scores of intelligence quotient (IQ) and adaptation development quotient (ADQ)alongside hair [Zn] and anthropometric measures in young Chinese children. They reported a positive correlation between hair [Zn] and ADQ in boys, but not IQ in either boys or girls.

Warthon-Medina *et a*l (8) performed a follow-up study in pre-school children (n=200) living in an economically deprived area in Lima, Peru who received multiple either micronutrient supplements (containing zinc) or Iron alone during infancy. Indices of motor development, IQ, working memory and executive function were measured. No long term impact of multiple micronutrient supplementation compared with iron alone were identified.

Older adults

Alghadier et al (9) used the Loewenstein Occupational Therapy Cognitive Assessment (LOTCA) battery. Serum [Zn], the serum concentration of iron ([Fe]) and copper ([Cu]) were measured. Participants were grouped according to their overall LOTCA score, normal, moderate decline and severe decline. Analysis of the data revealed a significantly lower serum [Zn] and higher [Fe] and [Cu] in participants with severe and moderately impaired cognitive performance ([Zn]: 48.9±3.4 and 65.4±1.6 µg/dL respectively) compared to normal ([Zn]: 78.3±2.5 µg/dL), p=0.01. Higher serum [Fe] and [Cu], and lower [Zn] were strongly associated with poorer cognitive performance, specifically in tests of long-term memory, motor praxis, vasomotor organisation, thinking operations, attention and concentration.

Markeiwicz-Zukowska (10) measured cognitive impairment using the Abbreviated Mental Test Score (AMTS) which is used to screen for signs of dementia, and emotional status was examined using the Geriatric Depression Scale (GDS). Serum [Zn] was higher (p=0.001) in subjects with unimpaired cognitive function (89 ± 20 µg/dL) compared to those with memory impairment (76 ± 22µg/dL). Serum [Zn] was positively correlated with AMTS ($p<0.001$) and negatively correlated with GDS

(p=0.006). Those with signs of depression had significantly lower werum [Zn] (77±17 μg/dL) compared to those without depression (89±22 μg/dL).

Taken together, these studies provide supportive evidence for the use of some aspects of cognitive function particularly those associated with memory, as functional markers of zinc status.

Zinc dependent proteins

The gene expression of the zinc binding protein, metallothionein (MT), and the zinc transporters (ZnT and ZIP families) were highlighted in the BOND review as emerging biomarkers of zinc status. These proteins are involved in the regulation of cellular zinc homeostasis, and thus have been postulated to respond to changes in dietary zinc intake. The search yielded 3 relevant studies investigating MT and/or zinc transporters (11, 13, 17).

Metallothionein and zinc transport proteins

A randomised control trial to examine the impact of zinc supplementation on the gene expression of MT and the zinc transporters was undertaken by Chu et al (11). Healthy adults were randomised to receive 22 mg elemental zinc per day for 21 days or no treatment. Blood was collected at intervals for 3 weeks and the ZIP, ZnT and MT mRNA was extracted and quantified from peripheral blood mononuclear cells (PBMC). Plasma [Zn] was also measured and dietary intake estimated from 3-day food records. Zinc supplementation did not change the gene expression of the zinc transporters or MT1A. Expression of MT2-A was higher in the zinc supplemented group compared to controls (p=0.025), and this transient upregulation occurred within 2 days of zinc supplementation but returned to values close to that of the control group by day 7. Plasma [Zn] was not significantly altered by supplementation. Multivariate analysis revealed that baseline dietary zinc intake was a significant predictor of all measured ZIP, ZnT and MT gene expressions, explaining 61% of the variance in gene expression. MT1-A and ZIP10 expression were both significant univariate factors

(p=0.002 and 0.045 respectively). Plasma [Zn] was not correlated with the gene expression of any of the proteins, nor did the change in gene expression following supplementation predict plasma [Zn].

In the study conducted by Sharif et al (12), elderly participants with low plasma [Zn] (<77 μg/dL), were randomly allocated to receive either a zinc supplement of 20 mg per day or a placebo for 12 weeks. RNA was isolated from lymphocytes and the expression of MT1-A and ZIP1 was measured along with plasma [Zn] at baseline and endpoint. Both MT1A and ZIP1 expression increased significantly in the zinc supplemented group compared to placebo ($p<0.05$). Plasma [Zn] also increased significantly in the supplemented group, and correlated with ZIP1 expression ($r=0.576$, $p=0.039$) post supplementation.

Giacconi et al (13) also studied the response to zinc supplementation in elderly participants with low plasma [Zn]s (<68.6 μg/dL). The study was designed to investigate a single nucleotide polymorphism (SNP) on the encoding region of the ZIP2 gene. Participants were grouped according their genotype (Leu+ or Leu-) and all were given a 10 mg/day supplement of zinc for 48±2 days. The expression of ZIP and ZnT proteins was measured in PBMCs, pre and post supplementation. There were some significant differences in the expression of zinc transport proteins at baseline between the two genotypes. In terms of changes in gene expression post supplementation, both genotypes exhibited a significant decrease in ZIP2 and ZIP3 mRNA, no significant changes in expression of the other transporters were reported. PBMC MT levels increased significantly post supplementation in all participants, with no differences according to genotype.

These studies contribute some valuable data to this emerging area of research. In the absence of pre-existing zinc deficiency there appears to be no change in the expression of the zinc transporters following supplementation (11), however, the level of transport gene expression may reflect the longer term dietary zinc intake levels. If zinc deficiency is present, there does appear to be an increase in ZIP1 and a decrease in ZIP2 and ZIP3 gene expression in response to supplementation, however it should be noted that baseline gene expression may vary depending on the presence of

SNPs. Total MT in PBMC and MT-2A gene expression increased following zinc supplementation, irrespective of baseline zinc status.

Oxidative Stress and DNA integrity

Zinc is a cofactor or structural component for proteins involved in DNA damage repair. Zinc deficiency therefore has deleterious effect on DNA integrity, resulting in an increase in the number of strand breaks. Two studies were identified that evaluate the impact of zinc supplementation on DNA damage in participants with low zinc status, both used the comet assay (12, 14) .

Joray et al (14) studied a group of Ethiopian women with low dietary zinc and high phytate intakes. Average baseline plasma [Zn] was 73.4 ±2.0 μg/dL and 37% of the women had values below the cut-off of 70.0 μg/dL. They received 20 mg zinc or placebo daily for 17 days. Blood taken at baseline and endpoint. Zinc supplementation did not significantly affect plasma [Zn], however the mean comet tail moment decreased significantly ($p<0.005$) post supplementation and there was a significant negative correlation between plasma [Zn] and tail moment at the endpoint ($r=-0.42$, $P=0.02$) but not at baseline.

In the study of elderly zinc deficient participants by Sharif et al (12) described previously, DNA damage was measured using the comet assay and also the cytokinesis-block micronucleus cytome (CBMN-Cyt) assay. Data from the comet assay revealed significant effect of time and treatment of zinc supplementation for both tail moment and tail intensity ($p<0.05$) which both decreased relative to baseline (7.53% and 8.76% respectively). Plasma zinc was inversely correlated with tail moment after 12 weeks of supplementation ($r=-0.219$, $p=0.045$). The CBMN-Cyt assay also revealed a significant reduction in DNA damage post supplementation relative to baseline.

These studies both provide further evidence that, in zinc deficient individuals, there is a measurable reduction DNA damage following zinc supplementation. This occured irrespective of a concurrent increase in plasma [Zn]. Further studies are needed to confirm this observation in different settings

and populations (infants, children, pregnancy), and the impact of other nutrient deficiencies that may affect the assay.

Taste acuity

Zinc is a component of gustin, a protein involved in taste perception, thus it has been postulated that zinc deficiency impairs taste acuity. Kim *et al* (15) studied haemodialysis patients, divided into 2 groups depending on their serum [Zn]; a zinc deficient group, (serum zinc <70 μg/dL) and zinc sufficient (serum zinc >70 μg/dL). Salt taste acuity and preference were determined by a sensory test using varying concentrations of NaCl solution. The mean threshold salt concentration for perception was significantly higher in the zinc deficient group (p=0.02), supporting an inverse relationship

between zinc status and taste acuity.

Conclusion

New data, published since January 2015, provide a valuable contribution to the evaluation of the potential and emerging biomarkers of zinc status in individuals, identified by the BOND expert zinc panel (2). In the absence of a "gold standard", potential biomarkers can only be compared against what is routinely used, and for which there is already a significant body of evidence, namely plasma (or serum) [Zn], growth and dietary zinc intake. Never-the-less, there are some exciting new possibilities that warrant further investigation.

Key points

1. **The assessment of zinc status in individuals is notoriously difficult, however recent comprehensive reviews have identified useful, potential and emerging biomarkers of zinc status**
2. **Newly published data support the use of hair [Zn] and indices of neurological function including memory and attention.**
3. **There is further evidence supporting the measurement of DNA integrity (using the comet assay).**
4. **Changes in the gene expression of zinc transport proteins in response to supplementation warrant further investigation.**

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Conflicts of interest. Nicola Lowe was a member of the BOND expert panel that published the recent review of zinc biomarkers, and also lead the EURRECA review of zinc biomarkers.

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Table 1. Summary of Biomarkers of Zinc status according the categories identified by the BOND Zn expert panel (summarised from King et al (2))

Recommended	Potential	Emerging	Not Useful
Dietary assessment	Hair Zinc	Nail Zinc	Zinc dependent enzymes
Plasma or serum zinc concentration	Urinary Zinc	Zinc-dependent proteins	Erythrocyte and leukocyte zinc
Stunting	Neurobehavioral function	Oxidative stress and DNA integrity	
		Zinc kinetics	
		Taste acuity	

Table 2. Summary of the publications included in this review.

Potential Biomarker	**Reference**	**Relevant outcome measures**	**Population**	**Evidence supports potential ***
Hair [Zn]	Gao *et al* (4)	Hair [Zn] plasma [Zn], anthropometry (height, weight and head circumference), Intelligence Quotient, Adaptation Development Quotient	Chinese school children aged 3-5 years, n=8102	0
	de Gier *et al* (5)	Hair [Zn], height for age Z score	Cuban children aged 4 to 17 years with (n=60) and without (n=120) helminth infection	+
	Koc *et al* (6)	Hair [Zn], serum [Zn]	Turkish adults with Alzheimer disease (n=22 women, n=23 men) and healthy adults (n=17 women, n=16 men)	+
	Bin Liu *et al* (3)	Hair [Zn], serum or plasma [Zn]	Meta-analysis of studies of adults with and without Myocardial Infarction	+
Neurobehavioural and cognitive function	Fuglestad *et al* (7)	Neurological development (Bayley Scales of Infant Development-III, elicited imitation tests), anthropometry (height and weight, weight for height z scores), serum [Zn], retinol binding protein, biochemical indices of iron, iodine, vitamin B12, selenium, vitamin D, vitamin A status	Infants aged 8-18 months, n=53, adopted into the USA from post-Soviet States (n=15), Ethiopia (n=26) and China (n=17).	++
	Gao *et al* (4)	Intelligence Quotient (Bitnet and Raven intelligence scales), Adaptation Development Quotient (behavioural adaptation scale), hair [Zn], plasma [Zn], anthropometry (height, weight and head circumference).	Chinese school children aged 3-5 years, n=8102	+

	Warthon-Medina *et al* (8)	Cognitive and executive function (Wechsler Preschool and Primary Scale of Intelligence (WPPSI) Verbal IQ sentences subtest, the Day-Night Stroop test, nine boxes memory test, theory of mind test, Brief Infant-Toddler Social Emotional Assessment (BITSEA))	Peruvian pre-school children, aged 3-4 years, n=200, follow up study to previous supplementation with multiple micronutrients (zinc,iron, folic acid, vitamins C and A) or Iron alone for 6 months during infancy (aged 6-19 months)	0
	Alghadir *et al* (9)	Cognitive performance (Loewenstein Occupational Therapy Cognitive Assessment battery, including orientation, visual perception, spatial perception, motor praxis, vasomotor organisation, thinking operations, attention and concentration), serum [Zn]	Healthy older adults aged 64-96 years, (n=100)	++
	Markeiwicz-Zukowska *et al* (10)	Cognitive and executive function (Abbreviated Mental Test Score, Geriatric Depression Scale)	Older adults aged 60-102 years, n=130	++
Emerging Biomarkers				
Zinc-dependent proteins	Chu *et al* (11)	MT mRNA MT (MT-1A, MT-2A), ZnT mRNA (ZnT 1, ZnT5, ZnT6, ZnT7), and ZIP mRNA, (ZIP1, ZIP3, ZIP7, ZIP8, ZIP10, ZIP14), plasma [Zn], serum CRP, dietary Zn intake	Healthy Adults aged 18-65 years, randomised to receive 22 mg elemental zinc per day for 21 days (n=20) or no treatment (n=19)	++
	Sharif *et al* (12)	ZnT mRNA ZIP mRNA, DNA integrity, dietary Zn intake, plasma [Zn]	Healthy Australian elderly adults aged 65-85 years, with low plasma [Zn] randomised to receive 22 mg elemental zinc per day for 21 days (n=20) or no treatment (n=19)	++
	Giacconi *et al* (13)	ZnT mRNA (ZnT1, ZnT6), , ZIP mRNA (ZIP 1, ZIP2, ZIP3, ZIP8), MT, Plasma [Zn]	European elderly adults with low plasma [Zn], aged 74.6 ± 68.7 years (n=1090), classified based on ZIP2 SNP Leu- (n=535) or Leu+ (n=555).	++

			All received Zn supplements, 10 mg per day for 48±2 days	
Oxidative stress and DNA integrity	Joray *et al* (14)	DNA integrity (comet assay), plasma [Zn]	Zinc deficient Ethiopian women, n=40. Randomly assigned to receive 20 mg zinc (n=20) or placebo (n=20) daily for 17 days	++
	Sharif *et al* (12)	DNA integrity (comet assay), plasma [Zn], ZnT mRNA ZIP mRNA, dietary Zn intake	Healthy Australian elderly adults aged 65-85 years, with low plasma [Zn] randomised to receive 22 mg elemental zinc per day for 21 days (n=20) or no treatment (n=19)	++
Taste Acuity	Kim *et al* (15)	Salt Taste acuity, serum [Zn], dietary assessment (3-day diet diary)	Adult end stage kidney disease patients receiving haemodialysis (n=77).	++

* 0 no evidence supporting biomarker, + evidence supporting biomarker, ++ strong evidence supporting biomarker